BIOLOGICAL STUDIES OF A LITTORAL MUSSEL, *HORMOMYA MUTABILIS* (GOULD) II. COMPARATIVE OBSERVATIONS ON CLEARING AND SQUIRTING ACTIVITIES IN TWO LITTORAL MUSSELS, *HORMOMYA MUTABILIS* (G.) AND *MODIOLUS AGRIPETUS* (IREDALE)[1]

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With 4 Text-figures

Introduction

In Hatakezima Island, there are found some places of the rocky substratum crowded by *Modiolus agripetus* (IREDALE) at the lower level of the intertidal zone near the low water tide mark. These are always well sheltered among the reefs, on the southern side of the reef area around the island, or sited on the east side of the island facing the stagnant water of the inner part of Tanabe Bay. *Modiolus agripetus* is distributed generally along the rocky shore around the Seto Marine Biological Laboratory in shallow tide pools or on the wall of bigger pools in the lower intertidal zone, forming rather small groups together with *Septifer bilocularis* (LINNÉ). However, this species of *Modiolus* forms usually much bigger colonies in such strongly protected places as shown in Hatakezima Island, where the substratum surface is very silty. Although it is very difficult to judge whether or not such a silty place is attractive to this mussel, it is quite evident that the mussel can well resist such a silty condition.

In contrast with this, *Hormomya mutabilis* (GOULD), another kind of littoral mussels, is distributed very differently. This mussel is found also in dense colonies on the rocky substratum nearly at the same level of the intertidal zone, but mainly in exposed places. This mussel may be found sparsely in the colony of *Modiolus* in silty places in small groups, but never in large numbers. Thus, frequently distributions of these two mussels are separated very clearly from each other in the same area, *Modiolus* in silty places in small groups, but never in large numbers. Thus, frequently distributions of these two mussels are separated very clearly from each other in the same area, *Modiolus* occupying the sheltered silty part of the area, while *Hormomya* occupying the

1) Contributions from the Seto Marine Biological Laboratory, No. 546.

exposed part with less silt. *Hormomya* can seemingly not stand the silty condition, and thus it can not compete with *Modiolus* in protected silty places. In other words, *Modiolus* must have some adaptive behaviours to this condition, which *Hormomya* lacks.

In order to survive and grow successfully in turbid water, *Modiolus* might have some more effective way to keep all undesirable or rejected particles away from it. For instance, MACGINITIE reported that squirting ability is common among bivalves living in a mud flat or turbid water (MACGINITIE, 1941). It is believed that morphological adaptation, together with powerful contractions of the adductor muscle, takes the main roles in separation of habitat, silty and with less silt, in two genera of oysters, *Crassostrea* and *Ostrea* in some estuaries (GREEN, 1968). Further, if the conjecture presented above is real, *Hormomya* will have to suffer smothering by crowded *Modiolus* in addition to suffocation by silty mud, as in an almost similar case occurred in quiet water of Santa Barbara Harbor, California between the two species of sea mussels, *Mytilus edulis* LINNÉ and *Mytilus californianus* CONRAD, of which the latter was smothered to death by the former (HARGER, 1970).

Now, regular outflow of water maintained rather steadily from the exhalant siphon of mussels will concern to keep away faecal pellets and some silty materials suspending nearby; the stronger the flow, the clearer the ambient water at least of the siphonal area. Thus first, some experiments were designed to measure the strength of water outflow in and compare it between *Modiolus* and *Hormomya* as a parameter of adaptability to the silty condition; the strength is then represented by two elements: the speed of water flow and the reach of faecal pellets.

Later, however, it was found that in addition to regular faecal pellets pseudofaecal matters were formed often in these two mussels. As noted already by many workers, ejection of pseudofaeces is universal in many ciliary feeding bivalves (OWEN, 1966), and this is done by either regular outflowing from the exhalant siphon or water squirting which will contribute much to clear the surrounding condition. For this reason, some observations and experiments were made additionally as to formation and ejection of pseudofaeces, especially as to water squirting.

All the observations and experiments were carried out at the Seto Marine Biological Laboratory in autumn and winter 1970–71.

I owe much to Dr. H. UTINOMI, the director of the laboratory, Dr. S. FUSE, Dr. C. ARAGA, Messrs. H. TANASE, S. SAKAI and Y. YAMAMOTO in obtaining materials and various facilities, Dr. S. NISHIMURA in looking for references, and particularly to Dr. T. TOKIOKA in planning experiments and preparing the present report. I am grateful to Mr. T. YAMAMOTO who identified an alga, and to Mr. S. SUZUKI of Tanabe City who kindly provided me 2 kinds of kaolin from Siga Prefecture. Special thanks are also due to Mr. H. MORINO and Miss J. KONDO for their helps in very many ways.

Materials and Methods

Both *Hormomya mutabilis* and *Modiolus agripetus* were collected from Hatakezima Island and the northern shore of the laboratory ground. As a rule, the mussels collected were kept alive intact in an aquarium with running sea water for at least 24 hours but not over 48 hours before they were put in use. This was to release them from any shock which might be given them at collection and to keep their physiological condition as natural and uniform as possible. But only in an experiment to measure the reach of faecal pellets, 23 of 50 specimens in each species had been kept alive in laboratory conditions for 25 days before they were threated finally. In this case they were fed with *Chlorella* sp. which had been confirmed successful in cultivating *Chlamys* (*Mimachlamys*) *nobilis* (Reeve) at the prefectural cultivation fisheries experimental station at Tanabe City.

Series of experiments were designed for two main ends, the first was to measure the speed of the water flow and the reach of ejected matters from the exhalant siphon to learn the comparative strength of the water flow in the two mussels, and the second concerned the clearing activity including pseudofaecal formation and ejection and water squirting, throughout the whole experiments, the water temperature was maintained in the range 14–16°C.

(1) *Measurements of the strength of waterflow from the exhalant siphon:* The strength of of waterflow from the exhalant siphon may be expressed by the transportation power of something of the flow. Several kinds of materials were thought of for this purpose; these must be small, with a certain weight, but not so heavy to be carried easily away by jet-water shot from the exhalant siphon. Small pieces of cork, plastic foams, chalk dust, etc. were tested, but unfortunately all this were proved to have some disadvantages when they were put into practice. Finally it was decided to cope with the problems to trace their own faecal pellets (inclusive of some pseudofaecal masses) expelled from the exhalant siphon. Then, in order to have enough faeces (or pseudofaeces) in compact masses, to be marked distinctively, at any time of experiments, the mussels were fed excessively with *Chlorella* about one hour before they were placed under experimental treatments. Both mussels show a trend in an unfed state to produce somewhat flat, slightly solid faeces of variable lengths, a little thicker in *Modiolus* and with a faint median longitudinal depression in *Hormomya* (Fig. 1), but well fed mussels will produce

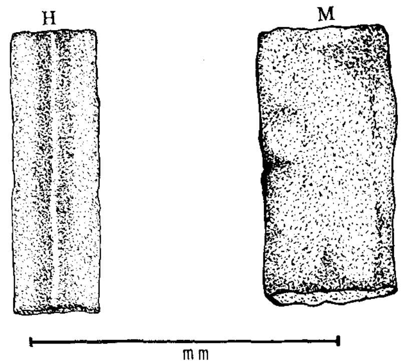

Fig. 1. Natural faecal pellets (unfed with *Chlorella* sp.) of *Hormomya* (H) and *Modiolus* (M).

much thicker and more mucid faeces.

Observations on these mussels fed well previously were made in quadrate white plastic plates (23 cm × 30 cm in extent and 5 cm high) filled with fresh sea water. A single specimen was placed completely submerged under the water in a plate at a corner with its posterior end diagonally towards the opposite corner, left for about two hours undisturbed, then the distance from the exhalant siphon to the farthest greenish spots marked on the plate bottom with faeces (or pseudofaeces) was measured and noted as the reach.

For the speed measurement of waterflow from the exhalant siphon, graphite dust was found to be the best material available at hand. This could be prepared very easily by scraping a pencil tip smoothly with a sharp knife or razor blade, or collected much more simply from a pencil-sharpener. Graphite dust will never sink down easily in sea water, first it will spread almost evenly over the water surface but in a few minutes gather to form numerous tiny clusters which are discriminated very easily in waterflow from the exhalant siphon. A single mussel was placed in a plate,, used in the previous observations but this time marked on the bottom with two concentric rings respectively 1 and 6 cm away from the centre. The mussel was laid with its posterior end at the centre as precisely as possible (Fig. 2a), and the amount of water was adjusted to cover just above the dorsal exhalant siphon. When the mussel begins to expel the water, clusters of graphite dust will be brought into the exhalant water current near the tip of the siphon, carried in the very surface layer of water from the first to the second ring, 5 cm apart from the first. Some clusters will be drifted in rather irregular directions, but some others nearly straight in the radial axis of circles; one of the latter will be marked and its drift can be timed by using a stop watch. Thus the speed is represented here by time in second spent for drifting for the first nearly 5 cm. The space of 1 cm from the posterior margin of mussels to the first ring was spared for finding out some cluster of graphite dust which was approximately of the same size for every mussel experimented with and likely to enter the waterflow at the most suitable spot to gain the maximum speed. The measurement was repeated three times on every specimen and performed with 15 individuals of each species.

(2) *Observations on clearing activity:* Formation of pseudofaeces was observed in the two mussels when they were supplied with excess *Chloralla*. As the pseudofaecal formation and ejection are apparently correlated closely with their tolerance to water turbidity, experiments were made with some turbidity-producing materials such as talc powder, natural silt collected from Hatakezima Island together with mussels, and a kind of kaolin from the town of Sigaraki in Siga Prefecture to induce these phenomena. Suspensions of these materials of different densities in fresh sea water, filtered through the finest gauze for plankton net which will allow only particles less than 50 in diameter to pass and maintained in temperature almost exactly the same as in the ambient water of treated mussels, were introduced into the mantle cavity of

mussels which were each laid vertically in a vial of a size to hold the mussel, with its anterior end down to the bottom of the vial and the posterior end up. Suspensions were released through a burette which was set so that its tip was dipped into the water in the vial but about 2 cm above the inhalant aperture of the mussel (Fig. 2b) and was

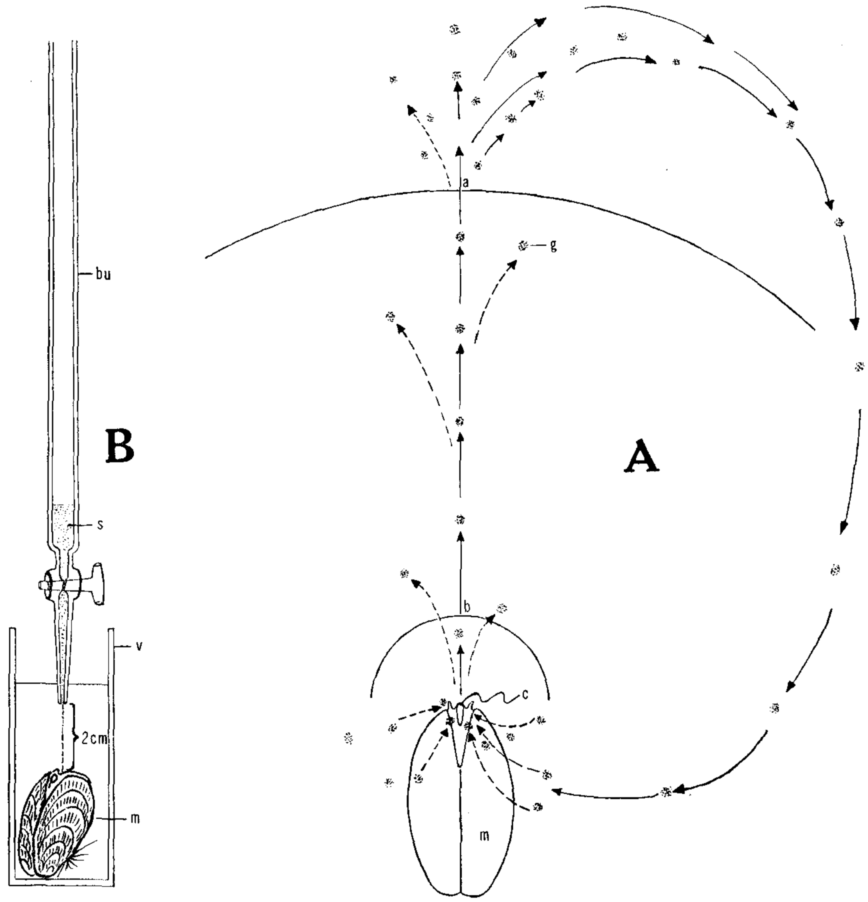

Fig. 2. Way of measuring the speed of exhalant waterflow (A) and way to inpour various suspensions into the mantle cavity (B). Solid arrows indicate an approximate main water current produced by a mussel and arrows in broken line indicate courses of clusters of graphite dust entering or escaping from the main current.
a—second concentric ring, b—first concentric ring, c—centre (ac—6 cm, bc—1cm, ab—5cm), g—cluster of graphite dust, m—mussel viewed from dorsal in Fig. A. bu—burette, m—mussel, s—suspension, v—vial in Fig. B.

adjusted so that the releasing rate was within a limit, confirmed empirically with clean sea water, not to give any mechanical stimulus to the mussel. When water squirting was induced, time to the first squirting evoked was measured. *Modiolus agripetus* usually armed with many long bristle-like conchiolin projections on the posterior portion and especially along the margin of its shell and very ofaten furnished further with an alga, *Gigartina intermedia* (SURINGAR), which will form a mass of thickly branch-

Table 1. Reaches of faecal pellets or pseudofaecal masses in two littoral mussels, *H. mutabilis* and *M. agripetus*, water temperature 14–16°C. Averages of shell lengths and reaches are given only to help a rough comparison between the two mussels.

No.	*Hormomya*				*Modiolus*			
	Mussels kept in laboratory conditions for 25 days		Mussels freshly collected		Mussels kept in laboratory condition for 25 days		Mussels freshly collected	
	Shell length in mm	Reach in mm	Shell length in mm	Reach in mm	Shell length in mm	Reach in mm	Shell length in mm	Reach in mm
1	12	15	14	11	11	46	11	40
2	15	16	16	42	20	72	15	54
3	19	22	17	24	20	73	17	97
4	19	110	20	70	23	127	21	120
5	21	91	22	41	23	130	21	50
6	22	27	23	95	24	38	22	100
7	24	135	24	18	25	47	22	138
8	25	33	24	53	25	107	23	115
9	26	58	24	75	25	125	23	168
10	27	20	25	60	26	114	23	140
11	27	130	25	10	26	164	24	106
12	28	25	26	70	27	129	24	130
13	28	77	26	145	28	53	25	124
14	28	62	26	80	28	225	25	155
15	28	191	28	50	30	78	26	140
16	29	66	28	76	30	114	26	120
17	29	80	29	75	30	85	27	137
18	29	71	29	75	31	267	28	140
19	29	103	29	67	31	182	28	130
20	30	187	30	62	32	144	28	155
21	32	137	30	82	40	261	29	124
22	35	81	30	70	40	260	29	153
23	37	145	31	130	42	219	29	220
24	…	…	31	90	…	…	30	170
25	…	…	32	72	…	…	31	152
26	…	…	32	83	…	…	32	120
27	…	…	35	102	…	…	35	210
Average	26 mm	81 mm	26 mm	68 mm	28 mm	133 mm	25 mm	130 mm
	Total shell length average 26 mm Total reach average 74 mm				Total shell length average 26 mm Total reach average 132 mm			

ed filaments more or less covering the in-and exhalant siphon, it was therefore necessary to remove the alga and shave off conchiolin projections for better observations.

Results

(1) *Strength of exhalant waterflow:* So far as deduced from the reach of faecal and pseudofaecal masses and the speed of the exhalant waterflow in the two mussels, the waterflow from the exhalant siphon is generally more powerful in *Modiolus* than in *Hormomya*, as is shown in Tables 1 and 2 and a graph (Fig. 3). No significant differences were discernible between the specimens freshly collected and kept alive for a long time (25 days) in laboratory conditions so far as the measurements of the feacal reach were concerned. Exactly saying, the size of faecal or pseudofaecal masses must be correlated in some extent with their reach, and in connection with this it should be noted faecal or pseudofaecal masses are usually considerably smaller in *Hormomya* than in *Modiolus*.

(2) *Ejection of pseudofaeces:* The cleaning or clearing behaviour differs much in ef-

Table 2. Speed of exhalant waterflow in *Hormomya mutabilis* and *Modiolus agripetus*. Water temperature fluctuated between 14 and 16°C.

No.	*Hormomya*		*Modiolus*	
	Shell length in mm	Time spent when clusters of graphite dust drifted for 50 mm* in sec	Shell length in mm	Time spent when clusters of graphite dust drifted for 50 mm* in sec
1	20	4	21	5
2	23	6	22	2.5
3	24	8	23	2.5
4	25	7	23	2
5	26	2	23	3
6	26	5	24	2.5
7	28	11.5	24	3
8	28	6	25	4
9	29	8	26	4
10	29	5	26	3.5
11	29	6.5	27	3
12	30	3.5	28	2
13	30	6	29	2
14	30	7.5	29	1.5
15	31	4	32	3
	Approx. shell length average 27 mm Approx. speed average 8 mm/sec		Approx. shell length average 26 mm Approx. speed average 17 mm/sec	

*Distance from b to a in Fig. 2. A.

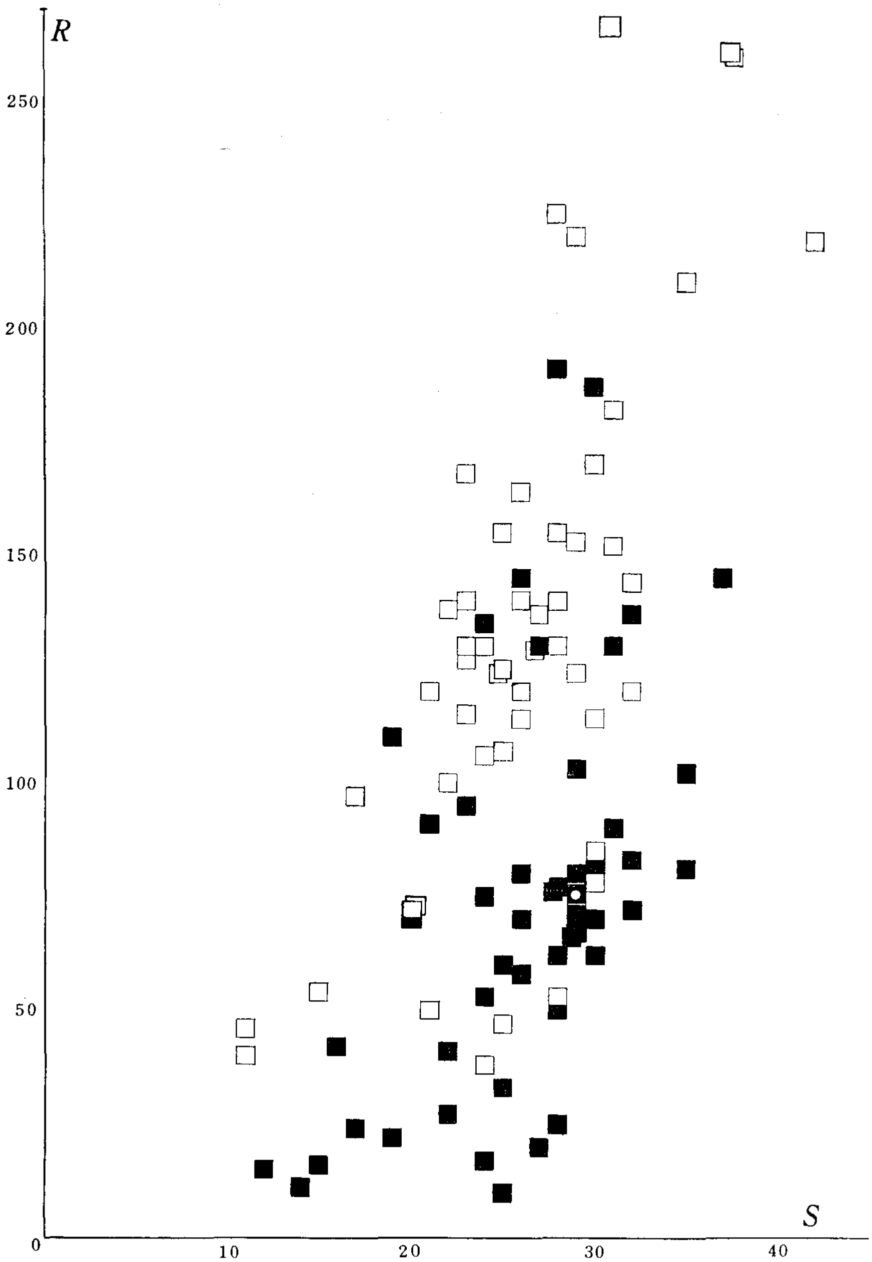

Fig. 3. Reaches of faecal pellets or pseudofaecal masses in *Hormomya* (solid square) and *Modiolus* (open square).
R—reaches in mm, S—shell length in mm, solid square with a white circle within shows the exactly same reach in two specimens of the same shell length in *Hormomya*.

ficiency in the two species. When suspension of turbidity-producing materials is poured into its mantle cavity, *Hormomya* reacts almost immediately but somewhat slowly. It will gently close shells together within 4–6 seconds and remain in this state till the inpouring of suspension ceases. Then a few minutes later it will open shells again and begin the clearing of the mantle cavity by forming pseudofaeces. Pseudofaeces are seemingly conveyed by ciliary movement of the inner mantle surface down to

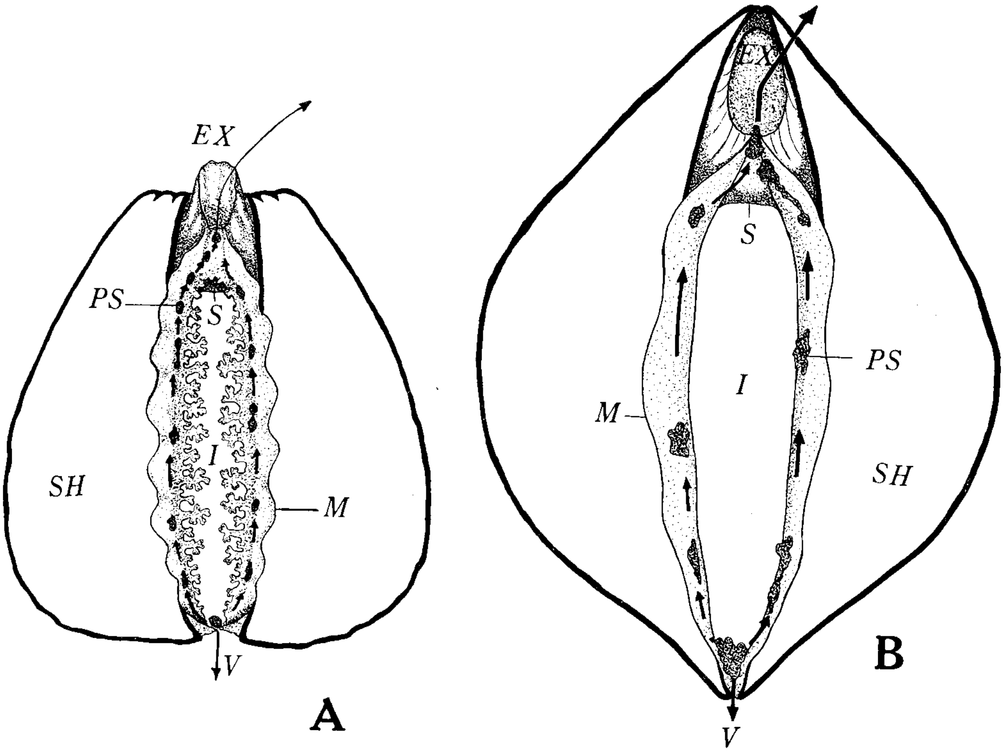

Fig. 4. Ejection of pseudofaeces in *Hormomya* (A) and *Modiolus* (B), shown semi-diagramatically. EX — exhalant siphon, I — inhalant aperture, surrounded with a number of sensory papillae in *Hormomya*, M — mantle edge, PS — pseudofaeces, S — septum separating the dorsal exhalant siphon from the ventral inhalant aperture, SH — shell, V — ventral region of the inhalant aperture, from where some pseudofaeces are extruded as shown by an arrow. Other arrows show the main route of pseudofaeces being conveyed dorsads and finally expelled from the ventral side of the exhalant siphon.

the ventral margins of the mantle (Owen, 1966); this was actually observed under an ordinary hand lens. Some small pseudofaecal masses were very possibly laden with mucus. Pseudofaeces are then carried posteriorly along mantle edges continuously one after another to the ventral region of the inhalant aperture, from there some big pseudofaecal masses, formed by aggregates of some small masses attaining there successively, will be extruded. Many small pseudofaeces are, however, carried further along either left or right mantle edge of the inhalant aperture to its dorsal region where the mantle edges of both sides are joined with each other for the first time to form a cleft leading to the exhalant siphon (Fig. 4a). Pseudofaeces are then driven along this cleft to the ventral border of the siphon and from there they are taken in the jet-current from the siphon. So far as I am aware, all the specimens of *Hormomya* experimented with behaved in the same manner, regardless of kinds, to talc powder, kaolin, and natural silt, or even to an excess amount of *Chlorella*, although in the last case pseudofaecal formation and ejection were maintained steadily without any closure of shells.

Modiolus behaves exactly in the same way as in *Hormomya* in formation and ejection of pseudofaeces, but the former differs distinctly from the latter in forming much larger pseudofaecal masses (Fig. 4b). The most significant of all is the efficiency of clearing behavious in *Modiolus*, which is enhanced extraordinarily by squirting ability in this mussel. It will squirt so violently at mechanical stimulations that a water drop may be shot out as far as 10 cm when the posteiror end of the mussel is exposed. When a small amount of some rejectable materials enter the mantle cavity, the shell will remain opened without any change in the span between the shell edges, although a very slight movement of mantle edges around the inhalant aperture shows that the animal is sensitive to this and then the pseudofaecal ejection will follow this. Further inpouring of rejectable materials in the mantle cavity at a constant rate will evoke squirting. Before squirting, most of specimens will extend mantle margins inwards from both sides till thin edges touch each other on the median line. At

Table 3. Induction of squirting in *Modiolus agripetus* (Iredale) by kaolin suspension of different concentrations. *Gram in 100 ml of fresh sea water)

Specimen no.	Shell length in mm	Concentration of kaolin suspension*	Volume of suspension poured in cc (less than)	Time to induce the first squirting
1	28	5	1	5 sec
2	38	5	1	15
3	30	5	1	10
4	30	5	1	10
5	29	5	1	2
6	39	1	2	28
7	32	1	1	18
8	45	1	1	26
9	36	1	2	16
10	29	1	1	12
11	35	0.1	3	2 min 4 sec
12	32	0.1	5	No squirt
13	30	0.1	2	1 min 10 sec
14	28	0.1	5	No squirt
15	33	0.1	5	2 min 25 sec

this moment, some specimens may even spread their shells a little more and this will be followed by sudden contraction of the posterior adductor muscle. Some psecimens may repeat squirting successively two or three times. There are many specimens which will do squirting without joining mantle edges prior to it. *Modiolus* did not behave differently to particles less than 50 μ in diameter of silt, talc powder, and kaolin.
(3) *Induction of squirting in Modiolus:* The relation between the squirting habit of *Modiolus* and the concentration and amount of kaolin suspension poured into its mantle cavity may be seen roughly in Table 3. To make sure that the squirting was not induced by a mechanical stimulus of flowing suspensions, every mussel was tested prior to experiments by inpouring fresh sea water into the mantle cavity even at a little bit stronger rate than the actual pouring rate of suspensions and it was found that there were not seen any discernible responses to the rate of water pouring in all treatments. It seems that at 0.1% concentration of kaolin suspension *Modiolus* shows its minimal irritation. *Hormomya*, which is devoid of a squirting ability, however, still reacts to the inpouring of 0.1% kaolin suspension in the same way, as shown previously, as to higher concentration.

Considerations

Throughout the results of observations and experiments, the lack of squirting ability and lesser strength of the exhalant waterflow in *Hormomya* are seemingly significant to bring about the separation of habitats in adults of *Hormomya mutabilis* and *Modiolus agripetus*. Possibly some features in their juveniles which must be very significant in making their habitats separate are still unknown, for instance the behaviours of spats of *Hormomya* in silty places and those of spats of *Modiolus* in both places, silty and with less silt.

Suspensions of talc powder, kaolin and natural silt induce squirting in *Modiolus* in addition to pseudofaecal ejection at concentration above 0.1%. However, a dense suspension of *Chlorella* sp., 6–8 μ in diameter, at a concentration of approximately 130 million cells/ml will never cause *Modiolus* and *Hormomya* to squirt or to manifest any reaction of irritation, though both mussels will reject surplus cells as pseudofaeces.

Green (1968, p. 151) states as "*Crassostrea* has some morphological adaptations which enable it to survive in silty conditions that would smother *Ostrea*. The left shell valve of *Crassostrea* forms a deep bowl which raises the edge of the shell above the surface of the substratum so that sediment does not enter the mantle cavity as easily as in the flatter *Ostrea*". This statement reminds us of the furnishing of the shell of *Modiolus agripetus* with conchiolin periostracum which might take part in preventing intake of coarse undesirable particles. The attachment of *Gigartina intermedia* (Suringar) on the posterior end of the same mussel is also very suggestive, because it branches thickly over the siphonal area of many specimens collected during the time of experiments. While, this alga is hardly found attached to *Hormomya mutabilis* in both the same silty habitat and adjacent unsilty areas.

Summary

1. The exhalant waterflow is much stronger in *Modiolus agripetus* than in *Hormomya mutabilis*.
2. Ejection of pseudofaeces is maintained in *Hormomya* by only ciliary movement, while in *Modiolus* by ciliary movement and muscle contraction resulting in squirting.
3. Squirting in *Modiolus* is inducible not only by intake of undesirable particles of a considerable size but also by inpouring of suspensions of smaller particles less than 50μ in diameter of concentrations above a certain level.
4. Higher clearing ability in *Modiolus* is seemingly correlated with its survival in silty habitats, while the distribution of *Hormomya* with less clearing ability is limited to exposed habitats with less silt.
5. Periostracum and attachment of *Gigartina intermedia* on the shell of *Modiolus* might be effectual to a life in silty habitats.

REFERENCES

Green, J. 1968. The biology of estuarine animals P. 151, Sidgwick and Jackson, London.

Harger, J. R. E. 1970. Comparisions among growth characteristics of two species of sea mussels, *Mytilus edulis* and *Mytilus californianus*. Veliger, vol. 13, no. 1, pp. 44–56.

MacGinitie, G. E. 1941. On the method of feeding of four pelecypods. Biol. Bull., vol. 80, no. 1, pp. 18–25.

Owen, G. 1966. The ejection of pseudofaeces. Distributed in Physiology of Mollusca, edited by Karl M. Wilbur and C. M. Yonge, vol. 2, p. 42, Academic Press, London.